RAD ENGINEERING REPORT (05/20/19)

INITIAL REVIEW ENGINEERING REPORT CBI: No

TERA: R19-0001 ENGINEER: Hollinshead/JAS (AFD Review)

SUBMITTER: Synthetic Genomics, Inc.
11149 North Torrey Pines Rd.
LaJolla, CA 92037

MICROORGANISMS:

Recipient/host (p. 16):

i. *Parachlorella* STR00012

Donor (p. 16):

i. loXP site derived from bacteriophage P1
ii. Green fluorescent protein "TurboGFP™"

GEM: The TERA is referred to as *Parachlorella* STR26155 (p. 16).

PV (CFU/yr): 6.0×10^{16} CFU/yr

Basis: Technical contact indicates that the ponds will reach a maximum density of 5×10^{7} CFU/ml, and that there will be approximately 12 batches maximum (process flow indicates 2 months but technical contact stated a maximum of 3 months per year, RAD assumes 3 months and 12 batches to be conservative)(see contact report). Submission states that the ponds in use will alternate between the two ponds. Based on this information, RAD calculates the PV to be:

Total PV = (12 batches/yr)*(100,000 L/minipond)*(1,000 mL/L)*(5×10^{7} CFU/mL)

Total PV = 6.0×10^{16} CFU/yr

Per the technical contact, weather conditions vary and only expects that operations will occur over 3 months, ideally occurring in different seasons - calculated PV will not occur year-round.

USE: This strain was developed to have virtually no discernable phenotypic differences relative to the recipient (i.e. starting) strain, but which possesses a nucleic acid signature and corresponding reporter protein to allow us to specifically track this strain in open-culture and in the environment (p. 16).

SUMMARY:

The specific insertions / deletions (and corresponding effects) are discussed in detail on pages 16-24 of the submission.

The submission states that the TERA will be used to collect real-world data on the potential for our algae to disperse, establish, and impact the local environment (p. 38).

The trial will take place at the Synthetic Genomics, Inc. – California Advanced Algae Facility (CAAF) in Calipatria, CA. The TERA will be cultivated in photobioreactors, then outdoor miniponds, and grown to a target density.

When the cell density in the minipond reaches the desired level, the TERA will be inactivated and disposed to an evaporation pond, before ultimately being disposed to a landfill.

NOTES AND KEY ASSUMPTIONS

The 1997 Generic Scenario for Biotechnology Premanufacture Notices was referenced in this IRER. In addition RAD referenced the May 12, 2000 technical policy memorandum on "Efficiency of Autoclaves for Laboratory-Scale Equipment" which assumes that for cases involving steam sterilization of laboratory scale equipment (<10 liters) in an autoclave, RAD will regard the potential for release of live microorganisms as negligible.

Per September 16, 2013 "Updating CEB'S Method for Screening-Level Estimate of Dermal Exposure," RAD updated the single-hand surface area from 420 cm^2 to 535 cm^2.

There are no past cases for this submitter or recipient microorganism (*Parachlorella*). The technical contact was called – see contact report.

The submitter indicates that once the field experiment has been terminated, all biomass will be inactivated by bleaching the cultures with at least 4 mL/L of 12.5% sodium hypochlorite for at least one hour prior to disposal. All equipment will be cleared of the microorganism (including sample containers, ponds, PBRs, etc.) by bleaching or autoclaving and will be discarded as necessary. Any pond spills will be contained within the secondary containment and treated with bleach. The liquid will then by disposed of into the evaporative pond at the CAAF site (p. 46)

Inactivation studies were performed on STR00010, STR00012, and STR26155. Experimental data showed that 2 mL/L of 4.0% sodium hypochlorite was sufficient to inactivate STR00010 after one hour. STR00012 and STR26155 were inactivated with 1 mL/L of 4.0% sodium hypochlorite after one hour with good mixing. All SGI protocols for inactivation utilize at least 4 mL/L of 12.5% sodium hypochlorite and a minimum contact time of 1 hour to ensure a total deactivated before disposal. Thus, standard SGI CAAF protocols apply greater than a 12.5-fold excess hypochlorite treatment (than that experimentally determined) to inactivate the subject strain providing a conservative treatment for algal cultures (p. 70).

The submitter also indicated that at the end of the toxicant contact time, the vessels were centrifuged to remove any extracellular toxicant, and the pelleted biomass was utilized to inoculate culture into fresh media. These cultures were incubated for one week before examining for growth. An inactivation method was deemed to be effective if after one week of growth, no viable cells were observed in the new culture vessels (p. 69). Therefore, the technical contact stated that they expect 100% inactivation, but indicated a minimum of 7-log inactivation efficiency (see contact report).

RAD assesses a 100% release scenario. After inactivation, the TERA will be sent to an on-site evaporation pond, and subsequently sent to landfill (p. 41).

Sporulation

Per submission, recipient and subject *parachlorella* strains grow as a uniform unicellular spherical cell. Alternate growth forms, such as filaments, colonies, spores, or cysts have never been observed and no flagella or sexual reproduction has been observed with this strain (p. 14); therefore, spore releases were not assessed.

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Manufacturing: Laboratory Propagation

Number of Sites/Locations: 1

Synthetic Genomics, Inc.
11149 North Torrey Pines Rd.
LaJolla, CA 92037

PROCESS DESCRIPTION:

The subject microorganism was created within the labs at SGI. The strain is then transported to the SGI La Jolla Greenhouse (within the same research park) in sealed secondary containers. There, the cultures are maintained and scaled prior to movement to the CAAF. Shipment of the subject microorganism will be made in clearly-labelled, sealed containers of approximately one to three liters. These will be further contained in secondary spill-proof containers and transported with enough bleach to neutralize the cultures in the case of a catastrophic failure (p. 44). The technical contact submitted 'SGI Algal biofuels culture scale up process and associated worker exposure' which indicated that the strain will be scaled up to five 40 L carboy then transferred to the Calipatria Research Station (see 'SGI Algal biofuels culture scale up process and associated worker exposure').

ENVIRONMENTAL RELEASE SUMMARY

Submission did not estimate releases during laboratory or greenhouse propagation. RAD assesses per standard methodology from the Biotech GS.

WATER: Negligible

Basis: No sources of release to this medium have been identified other than potential releases from residue in laboratory equipment. RAD's standard assumption for treatment of laboratory-scale equipment (<10 liters) is that releases are negligible (per the biotech GS; consistent with past biotech cases). The submission states that 'All laboratory biological waste is considered hazardous waste and will be disposed of into biological waste containers, then removed from the site and properly managed by a licensed hazardous waste vendor. The site holds both Federal and CAL/EPA registrations.'

AIR: Negligible

Basis: Typical operations to grow and harvest algae are not expected to generate significant quantities of aerosols containing the GEM. RAD's standard assumption is to consider air releases from this activity to be negligible (per the biotech GS; consistent with past biotech cases).

LANDFILL: Negligible

Basis: No sources of release to this medium have been identified other than potential releases from greenhouse residue in equipment and PPE. The 40 L carboys will be transported to the Calipatria, CA Research Station, any waste generated from equipment cleaning will go to Landfill, but is expected to negligible, compared to the other waste at Calipatria site.

INCINERATION: Not expected

Basis: No sources of release to this media have been identified (nor are they typically expected, per RAD generic scenario)

OCCUPATIONAL EXPOSURE

Submission does not provide worker exposure estimates for laboratory propagation. 'SGI Algal biofuels culture scale up process and associated worker exposure Non-CBI 09-May-2019' indicates completely enclosed transfer for scale-up, RAD is assuming some inhalation and dermal exposure in the laboratory/greenhouse setting as a worst case for potential sampling and monitoring growth.

Number of Total Workers: 2

Basis: The technical contact indicates that typically less than 2 employees are involved in this operation. RAD assumes 2 employees are potentially exposed.

Days/yr: 5

Basis: Algal biofuels culture scale up process and associated worker exposure document indicates a 7 week process to scale up but that worker exposure is only for 1 hour per inoculation & transfer. RAD assumes 5 exposure days.

INHALATION (bioaerosols):

From: Laboratory/greenhouse activities - culture transfer, preparing the inoculum, and monitoring the growth.

2 workers, 5 days/year
7-21 CFU/day

Basis:

Although air releases from the laboratory are expected to be negligible and inhalation exposures may be mitigated by use of standard aseptic techniques, RAD's biotech generic scenario recommends assuming some inhalation exposures in a laboratory setting as a worst case. The recommended method for estimating potential inhalation

exposures is to take the most applicable area monitoring data collected by NIOSH in a fermentation facility and multiply it by an estimate of the exposure duration. The NIOSH study listed the CFU concentration in a laboratory setting to be in the range from 32 to 103 CFU/m^3. Consistent with other recent GEM evaluations, the assumptions (and corresponding data) for exposure from 10 minutes of pipetting were used as analogous data to represent potential exposures to the microorganisms of this MCAN during laboratory operations.

- $[CFU]_{WA}$ = 32 - 103 CFU/m^3 (GS estimate for laboratory setting)
- I (inhalation rate)= 1.25 m^3/hr
- H = hours per day = 10 min = 0.167 hrs (RAD assumption). This is area monitoring data in contrast to personal breathing zone monitoring. In using area monitoring data, an assumption needs to be made about the duration the worker is proximal to the location of the bioaerosol. RAD typically assumes a short duration of time for the worker to be exposed to bioaeroosols during this part of the process.
- 5 days/yr (see above)
- 2 workers/site (see above)

Calculation:

$E_I = (I)(h)([CFU]_{WA})$ (per GS)

$= (1.25\ m^3/hr)(0.167\ hr/day)(32 - 103\ CFU/m^3)$

= 7-21 CFU/day

DERMAL:

Amount of Exposure:
2 workers, 5 days
5.5×10^6 CFU/day

Basis:
Biotech generic scenario. The potential dermal dose rate is the product of RAD standard dermal

exposure assessment factors and the CFU concentration in the appropriate process stream. For bench scale handling of liquids, the RAD standard dermal factor is <1.1 ml/day. This factor can be used with the concentration of the GEM (assumed to be equivalent to the final concentration during PBR fermentation, to estimate the dermal exposure:

- $[CFU]_B$ = 5.0 x 10^6 CFU/ml (unknown for laboratory/greenhouse propagation; RAD assumes this is equal to that for the PBR
- C = typical contact volume: <1.1 ml/day (revised RAD dermal exposure, 6/2000)
- 5 days/yr (see above)
- 2 workers/site (see above)

Calculation:

$E_D = ([CFU]_P)C$ (per GS)

= (5.0 x 10^6 CFU/ml)*(1.1 ml/day)

= 5.5 x 10^6 CFU/day

INITIAL REVIEW ENGINEERING REPORT CBI: No

TERA: R19-0001

PROCESSING/USE: Propagation in PBRs and Open Raceway Ponds

Sites/Locations: 1

Synthetic Genomics, Inc. – California
Advanced Algae Facility (CAAF)
250 West Schrimpf Road
Calipatria, CA, 92233

Days/yr: 98

Basis: Per submission, PBR batches will be inoculated every two weeks, grown for two weeks, and then utilized as seed to start the 0.1-acre ponds. (p. 48). The technical contact indicates that after the first batch, the other pond will be inoculated with the GEM from the first pond. The culture in the PBRs will only be used if there is contamination from the pond inoculation. Therefore, RAD assumes 14 days for PBR growth.

Batches will be produced every week, alternating between production ponds (p. 50). The technical contact indicates 12 batches, and RAD assumes 7 days per batch.

Therefore, RAD assesses 98 total days of operation (84 days of pond use and 14 days of PBR growth).

PROCESS DESCRIPTION

Seed stocks will be maintained in a dedicated grow room and transferred only between sealed containers during the scaling process. Once at least 100 L of seed has grown to a density of at least 1.0 g/L, the seed stock will be utilized to inoculate the 2,000 L and 4,000 L PBRs at a density of approximately 0.1 g/L. Once the PBRs reach a density of at least 1.0 g/L, they will inoculate one of the 0.1-acre ponds at a target operational

starting density of 0.1 g/L. These ponds will then run for one week each. At the end of a week of growth, the ponds will be deactivated and disposed (p.40).

PBRs and ponds have secondary containment in the form of a 24-inch berm that is lined with a mesh reinforced, puncture resistant, UV-resistant material. The berm has an effective footprint of 1 acre and can hold the approximately 5x the capacity of the two 0.1-acre L ponds plus all PBRs, in the highly unlikely scenario of complete primary containment failure (p. 45).

The submitter will regularly sample multiple sample types from a variety of sites (e.g. bioaerosols, trap ponds, CAAF production ponds, local environmental sampling) to provide data on the potential release of the engineered alga from the experimental ponds. The submitter will conduct active monitoring for one week prior to the start of open engineered alga cultivation, during the entire course of the experiment, and for 2 weeks following termination of the engineered alga ponds. During this active monitoring period, one type of endpoint will be the five 350 L “algae-trap” ponds established to help assess the dispersion capability of the subject organism. Additionally, the submitter will sample regularly from all other ponds on site that are in active use and assay for the presence and abundance of the subject strain. Lastly, regular bio-aerosol samples will be collected and similarly assayed for the presence and abundance of the subject strain. Both during the active monitoring, and for one year following first inoculation, the submitter will continue to carry out passive monitoring consisting of monthly sampling from established environmental stations (p.40).

Samples will be collected daily for the CAAF Lab to perform growth measurements. Briefly, these measurements will include optical density (OD730), ash-free dry weight (AFDW), photosynthetic efficiency (PAM), total organic carbon (TOC), fatty acid methyl ester composition (FAME), microscopic analysis and metagenomic analyses. Excess samples will be disposed of in 0.5% sodium hypochlorite. The culture will be inoculated with media containing nitrogen, phosphorus, and trace minerals. (p. 40)

At the end of each experiment, the ponds will be deactivated-in-place with at least 4 mL/L of a 12.5% sodium hypochlorite solution before disposal in the site’s evaporation pond. (p. 41)

Clean-in-place procedures are utilized for cleaning ponds at the CAAF site. At the conclusion of an experiment, ponds are scrubbed along the sides with brushes to remove any films that may have formed over the course of an experiment. Then, ponds are dosed with 4 mL/L of 12.5% sodium hypochlorite and thoroughly mixed with the in-pond paddlewheels. After at least one hour, and after complete mixing, the ponds are then pumped directly to the on-site evaporative disposal pond via a dedicated line. (p. 70).

ENVIRONMENTAL RELEASE SUMMARY

To ensure that the subject microorganism is completely removed from the test site after the experiment has been completed, all liquid biomass will be treated with 4 mL/L of 12.5% sodium hypochlorite for at least one hour prior to disposal. This dose is 12.5-fold greater than the experimentally determined effective dose for killing both recipient and subject strains. Scale up vessels, including Fernbach flasks and carboys, will be treated with bleach to neutralize the microorganism before dumping down the drain to the evaporative pond. Carboys will be cleaned and autoclaved for reuse. 0.1-acre ponds will be deactivated in place with bleach before disposal into the evaporative pond. Samples that have been collected from the site will be neutralized by treatment with 4 mL/L of 12.5% sodium hypochlorite for a minimum of one hour before disposal (p. 45).

WATER:

Amount: negligible

Basis: The submission states that the CAAF is a zero-discharge site for wastewater. Post inactivation, PBRs are pumped to an onsite evaporation pond (p. 49). Evaporated biomass is subsequently sent to landfill.

LANDFILL (from evaporation pond):

Per submission, all process liquid waste is piped to an evaporation pond with a total capacity of 8.6 acrefeet (AF). The pond is permitted by the California Water Quality Control Board Region #7. The pond was designed to comply with Federal, State and County construction standards. Quarterly Reports on the evaporation pond physical

integrity, chemical composition and water levels are provided to the State. (p. 42)

Evaporated salt waste material that is >50% water can be shipped via licensed hauler in lined dump trucks to a licensed Class-II landfill for disposal (lined to contain liquids). However, the preferred means of disposal will be to allow the material to dry below 50% water, and when the dried material passes the EPA “paint filter test” it will be shipped via a licensed vender in unlined trucks to a licensed Class-III landfill. A Special Waste Profile has been approved by a local landfill. (p.42)

1) From: PBR cleaning

Amount: negligible

Basis: Per technical contact, PBR cleaning will happen approximately twice per year (see contact report). Bleach solution will be run through the equipment to inactivate the GEM. The submission indicates that scale up vessels will be dumped to the evaporative pond after treating with bleach (p. 45).

The PBR size is 2,000 L for three PBRs(6,000 L), and the algae concentration from the PBR is 5×10^6 CFU/ml. The CFUs involved in this process are several orders of magnitude lower than the pond process (5×10^7 CFU/ml; 100,000L) (see contact report). Therefore, the releases from PBR cleaning are expected to be negligible compared to pond termination and pond cleaning. RAD also assesses 2% residual for equipment cleaning as a conservative estimate.

2) From: Pond/Equipment Cleaning

Amount:

1.2×10^8 CFU/yr
1.0×10^7 CFU/day over 12 days/yr

Basis: Per submission, after all the inactivated biomass(4 mL/L of 12.5% sodium hypochlorite) has been removed, any areas where visible biomass has adhered to the sides of the pond will be sprayed with 4.0% sodium hypochlorite and scrubbed from the side of the liner with brushes. After cleaning has concluded, brushes will be decontaminated with 4.0% sodium hypochlorite (p. 52). RAD uses the total PV and RAD 2% residual model to calculate these releases.

- 6.0×10^{16} CFU/yr (PV, see calcs above)
- Equipment cleaning and 12 pond batches/yr and 1 release day/harvest
- 2% multiple vessel residual model
- Inactivation efficiency of 7-log (see contact report).

LR = (PV)(1 - 0.9999999)(2% equipment residual)

= $(6.0 \times 10^{16}$ CFU/yr)(1 - 0.9999999)(0.02)

= 1.2×10^{8} CFU/yr

Per day:

= $(1.2 \times 10^{8}$ CFU/yr)/((12 batches/yr)* (1 release day/batch))

= 1.0×10^{7} CFU/day

3) From: Unused PBR Biomass Termination

Amount: negligible

Basis: Technical contact indicated that the PBR will be run continuously, and will be used to inoculate the pond as necessary (technical contact). RAD assumes that any biomass that may not be pumped directly into the pond will be inactivated then dumped

into the evaporation pond. Given the PBR total volume (6,000 L), expected growth period (2 weeks), and algal concentration(5×10^7 CFU/ml), the releases were orders of magnitude below the releases due to pond termination and pond cleaning. Therefore, the releases from any discarded biomass from the continuous PBR operations are expected to be negligible compared to pond termination and pond cleaning.

4) From: Pond Termination

Amount:

5.9×10^9 CFU/yr CFU/yr
4.9×10^8 CFU/day over 12 days/yr

Basis: After growth is complete, the ponds are inactivated and the waste sent to the evaporation pond (see Release Summary above). RAD assesses 100% release scenario.

- 6.0×10^{16} CFU/yr (PV, see calcs above)
- 98% of PV disposed (assuming 100% release scenario: 100% - 2% equipment cleaning)(Note, aerosol releases are several orders of magnitude lower than equipment cleaning and separation wastes and were not considered here).
- 12 batches/yr (technical contact)
- Inactivation efficiency of 7-log (technical contact)

$LR = (PV)(1-0.9999999)(98\%)$

$= (6.0 \times 10^{16}\ \text{CFU/yr})(1 - 0.9999999)(0.98)$

$= 5.9 \times 10^9$ CFU/yr

$= (5.9 \times 10^9\ \text{CFU/yr})/(12\ \text{days})$

$= 4.9 \times 10^8$ CFU/day

AIR:

1) From: Bioaerosol emissions

Amount:

6.0×10^7 CFU/yr
7.1×10^5 CFU/day, over 84 days/yr

Basis: Air releases can occur from aerosols generated from agitation due to sparge gas or paddlewheels. Currently, RAD does not have methodology for estimating these types of releases; therefore RAD estimates this potential release using the methodology described in the Biotech GS for fermentor exhaust gas.

- 12 batches/yr (tech contact)
- 7 days/batch in pond (p. 44)
- 84 days/yr (12 bt/yr x 7 days/bt) (technical contact and p. 44)
- 100,000 L pond Broth Volume (p. 39)
- Aerosolization factor (dimensionless factor indicating the proportion of CFU-containing aerosol particles in the size range of 1 to 10 microns formed per initial number of cells in the liquid volume considered) of 1×10^{-9} (RAD GS default)
- 5×10^7 CFU/mL (max final broth concentration per technical contact)
- 0% Removal Efficiency (no engineering controls are employed to reduce air emissions)

Calculations (based on std. RAD methodology for fermentor exhaust gas):

$AR_{FO,\ total} = ([CFU_B])(AF)(1-n_R)(V_B)$

$AR_{FO,\ total}$ = (5 x 10^{7} CFU/mL)(1 x 10^{-9})(1-(0))
(100,000 L/pond)(1000 ml/L)

$AR_{FO,\ total}$ = 5 x 10^{6} CFU/batch

Annual total

= (5 x 10^{6} CFU/batch)(12 batch/yr)

= 6 x 10^{7} CFU/yr

Per day:

= (6 x 10^{7} CFU/yr per TERA)/(84 days/yr)

= 7.1 x 10^{5} CFU/day

2) From: Fugitive Emissions During Sampling

Amount: Negligible

Basis: Per the 1997 Biotech GS, potential fugitive air releases from sampling have shown to be either undetectable or several orders of magnitude lower than those from fermentor off-gas, centrifugation, and filtration. Therefore, compared to other air emission sources, sampling air emissions are considered to be negligible.

INCINERATION: Not expected

Basis:

The submission indicates that waste will be disposed to the evaporation pond and subsequently landfilled. This is consistent with the 1997 Biotech GS, which does not specify any expected releases to incineration.

OCCUPATIONAL EXPOSURE

Number of Total Workers: up to 20

Basis: The submission states that there will be three to four workers involved in the initial application and three to four workers involved in the subsequent activities (e.g. sampling, pond monitoring.) The submission provided worker estimates in the following table (Table G2):

Worker Activity	PPE	# of Workers Exposed	Maximum Duration (hr/day)	Maximum Duration (day/yr)
Scale-up of cultures	Proper PPE[a]	3-4	4	52
Inoculation of ponds		3-4	4	52
Sampling of ponds		3-4	1	365
Sample processing (lab)		3-4	2	365
Experimental termination		3-4	4	52

Source: Table G2
a-Proper PPE includes: gloves, safety glasses, long pants, and steel-toed shoes. (p. 43)

Note that exposures were assessed for up to 20 workers, assuming that the same worker does not perform more than one activity. Therefore, the number of workers exposed may be less depending on whether the same workers perform multiple activities.

Days/yr: up to 98 (12 batches with 7 days/batch plus 14 days per year for PBR scale-up) (per technical contact and p. 44)

PPE: The submission indicates that proper PPE includes gloves, safety glasses, long pants, and steel-toed shoes. (p. 43)

INHALATION (bioaerosols):

1) From: Sampling Near Paddlewheels

Amount of Exposure:

Up to 4 workers (sampling in table).

60 to 411 CFU/day, 84 days/yr

Basis:

RAD’s 1997 Biotech Generic Scenario includes area monitoring data collected by NIOSH in a fermentation facility. The GS recommends estimating potential inhalation exposures by taking the most

applicable monitoring data and multiplying it by an estimate of the exposure duration. RAD does not have methodology for estimating exposures from aerosolization of liquids near paddlewheels. Therefore, RAD conservatively uses data near a centrifuge.

- $[CFU]_{WA}$ = 47.6 CFU/m^3 (NIOSH average of geometric means at centrifuge) to 329 CFU/m^3 (NIOSH max at centrifuge, per GS – unknown whether indoor or outdoor)
- 84 days/yr (7 days/batch for pond operation x 12 batches)
- I = 1.25 m^3/hr
- H = hours per day = 1 (Submission)

Calculations (based on std. RAD methodology):

$E_I = (I)(h)([CFU]_{WA})$ (per GS)

$= (1.25\ m^3/hr)(1\ hr/day)(47.6 \text{ to } 329\ CFU/m^3)$

= 60 (avg) to 411 CFU/day (worst case)

2) From: Inoculation and Scale-Up, PBR sampling, Sample Processing and Experimental Termination

Amount: Negligible

Basis: Per the 1997 Biotech GS, potential fugitive air releases from general sampling are shown to be either undetectable or several orders of magnitude lower than other sources. Therefore, compared to other air emission sources (paddlewheels), inhalation exposures from inoculation and scale-up, PBR sampling, sample processing in the laboratory, and experimental termination are considered to be negligible.

DERMAL:

1) From: Laboratory Work, Scale-up and Daily Sample Processing

Amount of Exposure:

20 workers/site
2.0×10^7 to 5.5×10^7 CFU/day, up to 98 days/yr

Basis:

The submission indicated that approximately 100 mL of sample will be taken from the pond every day (p. 43). RAD also assumes samples will be taken during PBR scale-up. Per the biotech GS, the potential dermal dose rate is the product of RAD standard dermal exposure assessment factors and the CFU concentration in the appropriate process stream.

- $[CFU]_B = 5 \times 10^7$ CFU/mL (max broth concentration, per technical contact)
- C = liquid transfer - 1 hand, the RAD standard dermal factor is (535 cm^2/day)(0.7 to 2.1 mg/cm^2)(1 g/1000 mg)(1 mL/g) = 0.4 to 1.1 mL/day (RAD assumes 1 hand transfer for sample analysis – note revised hand surface area per 2013 guidance – see Key Notes and Assumptions)
- 98 days/yr (total PBR and pond operation days)

Calculation:

$(5 \times 10^7$ CFU/mL)(0.4 to 1.1 mL/day)

$= 2.0 \times 10^7$ to 5.5×10^7 CFU/day

Non-CBI CONTACT REPORT **(TERA: R19-0001)**

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Submitter: Synthetic Genomics, Inc.

DATE: April 16, 2019

Person Contacted: Dr. David Hanselman / Dr. Jay McCarren
Affiliation: SGI Senior Director

Telephone: 858-433-2218 (California)

Caller: Jason Sese
Affiliation: ERG for RAD

Q: What is the production volume in CFUs? What is concentration in CFU/ml?
A: The pond OD will be grown from = 0.1 to 1 generally within a one week span. OD = 0.1 generally corresponds 5E+6 cells/ml, and 1 corresponds to 5E+7 cells/ml. These are presented generally as equivalent for our purposes to CFUs.

Q: How many batches per year?
A: Batches will be dependent on the environmental conditions. We believe we will only be running for 3 months (maximum) out of the year, with the ponds running for 1 week each. Potentially 1-month campaigns – we would like to perform the experiment during different seasons.

Q: Are all of the PBRs used per run, and per batch? How often will they be cleaned?
A: Likely only one of the PBRs will be used for the GEM. The PBR will run continuously, and will be used to inoculate the pond as necessary (RAD assesses the 4,000L PBR, in agreement with the technical contact). After the first batch, the active pond will typically be used to inoculate the second pond. The PBR culture will only be used to inoculate the pond if a fresh batch is needed. The PBR may be cleaned twice per year. We will send bleach solution to inactivate the GEM, and then dispose and replace the tubing.

Q: During the inactivation studies, how is “growth” observed? (p. 69) Is there a limit of detection? Minimum cell kill? 6 log?

A: We assume 100% inactivation, because of the 1-week growth period. Since all of the GEM is inactivated and we start with 10^7 cells, we assume a minimum of 7- log reduction.

Submitter: Synthetic Genomics, Inc.

DATE: May 9, 2019

Person Contacted: Dr. David Hanselman
Affiliation: SGI Senior Director

Telephone: 858-433-2218 (California)

Caller: Whitney Hollinshead
Affiliation: RAD

Q: How many workers will be exposed at the La Jolla facility?
A: The strain will be grown first at the La Jolla facility in our research greenhouse in hang bags. Then the strain will be transported to the Calipatira location. I estimate 2 workers will be handling the strain.

Q: The previous telephone contact log stated that the PBRs will be run continuous.
A: For the engineered strain, it will be more similar to a batch use. We will first grow the strains and pump into the pond. It will likely be only 1 inoculation, perhaps two. The technical contact later specified that the PBRs are expected to be run continuously. We will clean the PBR directly after culture with engineered strain and will then use the PBRs for experiments with other non-engineered strains. **Note: The technical contact will submit a flow chart of expected use of the GEM.**